

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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Supplementary Appendix

Clinical, Cognitive, and Biomarker Changes in the Dominantly Inherited Alzheimer Network

Running title: Cross-sectional estimates of the rate and order of Alzheimer's disease changes measured in the DIAN study using the estimated years to onset

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Table of Contents

DIAN SUPPLEMENTARY METHODS:	3
DIAN Recruitment and Eligibility.....	3
DIAN Inclusion and Exclusion Criteria	4
Genetic Testing	5
Brain Imaging Methods.....	6
Analyses	7
DIAN Parental Age of Onset Determination	8
Figure S1: Parental Age of Onset Form	9
Figure S2: Comparison by EYO with 95% CI	11
Table S1: DIAN participant characteristics	12
Table S2: Comparison of <i>PSEN1</i> to <i>PSEN2</i> and <i>APP</i>	13
Table S3: Interaction terms of model.	14
Table S4: Number of participants by EYO	15
Author contributions:	16
Supplementary References.....	17

DIAN SUPPLEMENTARY METHODS:

DIAN Recruitment and Eligibility

Recruitment:

DIAN participants were recruited from DIAN performance sites. Additional participants were recruited through local initiatives at the individual performance sites and through broader efforts (e.g., <http://dian-info.org/>, <http://www.alzforum.org/new/detail.asp?id=1967>, <http://www.alz.org/trialmatch> and <http://www.dianexpandedregistry.org/>). Identification of additional individuals was facilitated through DIAN's interaction with NCRAD, Alzforum, the Alzheimer's Association, NIA/ADEAR, and recruitment letters enclosed in genetic test results from Athena Diagnostics to physicians ordering tests.

Most eligible DIAN participants have not been clinically tested for ADAD mutations. To avoid indirect notification of mutation status, all potential DIAN participants were invited to the research program, regardless of mutation status. The participants without mutations contribute to the study as sibling controls.

Eligibility Screening

Prior to enrollment, each participant is interviewed to determine whether they meet eligibility criteria (below). Source documentation was provided to confirm the presence of a known mutation in the pedigree. The participants could be symptomatic or have diagnosed AD, and most (66%) in this cohort were asymptomatic.

Participant Assessment

All participants which completed assessments by the first data freeze were quality control checked and included in the analysis (n=128). None were excluded. Only baseline assessments were included in the analysis because there were few longitudinal follow-up assessments completed. Both mutation carriers and non-carriers, continue in the DIAN study for longitudinal follow-up every three years unless the participant has cognitive symptoms or is within three years of the EYO, in which case the follow-up is annually.

DIAN Inclusion and Exclusion Criteria

Inclusion Criteria

§ Written informed consent obtained from the participant and collateral source prior to any study-related procedures.

§ Participant is aged > 18 years inclusive **and** the child of an affected individual (clinically or by testing) in a pedigree with a known mutation for ADAD.

§ Participant is cognitively normal or if demented does not require nursing home-level care.

§ Participant has identified two persons (minimum of one) who are not their full-blooded siblings who can serve as collateral sources for the study.

§ Participant is fluent in a language approved by the DIAN Coordinating Center at about the 6th grade level or above

Exclusion Criteria

§ Participant has a medical or psychiatric illness that would interfere in completing initial and follow-up assessments.

§ Participant requires nursing home-level care.

EXCLUDED MEDICATIONS

DIAN has no exclusionary medications.

Genetic Testing

Each individual was screened for their family-specific mutation using PCR-based amplification of the appropriate exon followed by Sanger sequencing. Primers were designed to target each exon and at least 50 bp of 3' and 5' flanking intronic sequence of PSEN1, PSEN2 or APP (primer sequences are available from the authors upon request). Sequencing was performed using ABI Big Dye version 3.1 (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Sequencher software (Gene Codes, Ann Arbor, MI). For individuals who were mutation negative we also performed pooled Next Generation Sequencing to confirm that there were no mutations elsewhere in any of the FAD genes.¹ Apolipoprotein E genotyping was performed using Taqman genotyping technology as previously described.² DNA fingerprinting was performed with the Cell ID kit, using short tandem repeat (STR) analysis of 10 specific loci in the human genome, nine STR loci and Amelogenin for gender identification (Promega #G9500, Madison, WI). Quality assurance for each sample included comparison of results for DNA mutation, APOE genotype and DNA fingerprint for two independently collected samples from each individual. All sample pairs demonstrated 100% concordance.

Brain Imaging Methods

The volumetric scan consisted of a 5-6 minute 3D T1-weighted image (i.e. MP-RAGE) with 1.0x1.0x1.2 mm resolution. Amyloid imaging was performed with [11C]PIB-PET, acquired with a 30 minute dynamic (6 x 5 minute frames), 3D acquisition, beginning 40 minutes after a bolus injection of 15 mCi of PIB. Metabolic imaging with [18F]FDG-PET was performed per standard protocols, including a 20 minute dynamic (4x5 minute frames), 3D acquisition beginning 40 minutes after a bolus injection of 5 mCi of FDG.

The T1-weighted MR scans from DIAN participants were processed through the FreeSurfer image analysis suite Version 5.0. FreeSurfer involves cortical surface reconstruction and volumetric segmentation.³ The processing routine includes segmentation of the subcortical white matter and deep gray matter volumetric structures, extraction of the cortical surfaces, and parcellation of cortical regions. Output volumes were then corrected for total intracranial volume using established methods.⁴ PIB-PET frame-to-frame motion correction and PET-MR alignment was accomplished through standard registration techniques using in-house software.⁵⁻⁷

FDG and PIB-PET images were then transformed into each individual participant's MRI space. For each FreeSurfer region-of-interest, standardized uptake value ratio (SUVR) was calculated using a hand-drawn reference region encompassing the brainstem. The statistical analyses were also compared using a FreeSurfer cerebellar grey matter reference region and the findings were consistent. For image display of PIB-PET, voxel-wise estimates of fibrillar amyloid deposition at specific ages of onset were calculated in R using Loess,⁸ a locally weighted polynomial regression method. Prior to Loess fitting, each subject's SUVR image was registered to a common atlas target.⁹ SUVR images were then resampled to 1mm isotropic voxels and smoothed with a 5mm FWHM Gaussian filter. A 1st degree Loess curve was then fit for each voxel in atlas space.

Analyses

Statistical analyses were implemented using PROC MIXED (version 9.3, SAS Institute, Inc. Cary, North Carolina).¹⁰ Treating each marker as a continuous scale, a linear mixed model was used to model each marker as a function of EYO, mutation status (carrier vs. non-carrier), and apolipoprotein E (ApoE) status (presence vs. absence of an $\epsilon 4$ allele). ApoE4 status, instead of the number of $\epsilon 4$ alleles, was included in the model because very few participants had two $\epsilon 4$ alleles. All possible 2-factor, 3-factor interaction terms along with 2nd and 3rd order EYO terms were examined to reach a final model that fit the data well for each marker (Supplementary Table 3). Due to the fact that some participants were recruited from the same family, a random effect was also included in the mixed model to account for the family affiliation. Approximate Student t-tests derived from the model were used to determine whether marker values differed between mutation carriers and non-carriers at certain age points (Supplementary Table 4) after adjusting for the correlation among family members. Statistical significance was defined by $P < 0.05$. Scatterplot smoothing was done by Loess, a locally weighted polynomial regression method.⁸ Fitted Loess curves with a 95% confidence interval band generated by code written in the R language¹¹ (version 2.8.1) were presented. Individual values were not displayed on graphs to protect the confidentiality of mutation status of participants (e.g., a participant that does not know their mutation status could deduce mutation status from individual values by EYO). Figure 1 was generated with the same final models using the standardized difference between mutation carriers and non-carriers as a function of EYO, i.e., the predicted difference at each EYO divided by the SD for clinical, cognitive, imaging and biochemical measures.


DIAN Parental Age of Onset Determination

The DIAN Parental Age of Onset form is included as Supplementary Figure 1. Information regarding the affected parent's age at onset (AAO) is obtained from the participant, the collateral source, and/or other informants who may know the parental history of disease using a semi-structured interview tool. At the initial study visit, the site clinician documents the parental AAO after discussion with all informants and review of all available sources of information that may be useful (e.g. medical records and peripheral family members). AAO of the affected parent is calculated by subtracting the year of onset of the first progressive symptom (e.g. memory/cognition, motor or behavior) from the affected parent's year of birth.

Parental AAO undergoes clinical quality control procedures to ensure consistent reporting of last known normal, first progressive symptom onset, dementia onset, and death. The AAO data is reviewed by the clinical monitor, DIAN Genetics Core, and DIAN Global Clinical Coordinator to ensure accuracy in calculation and consistency of reporting across sites. If the accuracy of the reported AAO is determined to be uncertain during the QC review process, sites are contacted to discuss the data collection process and the reliability of the informant. Sites may then be asked to seek additional source documentation of the parental history of disease (e.g. medical records). Also, the Alzheimer's Disease Cooperative Study (ADCS) and Washington University Central Neuroimaging Data Archive (CNDA) data management teams perform quality assurance checks within the respective databases to detect inconsistency in calculation and reporting of the AAO within individual DIAN participant records and across all members of the same family.

Although these quality control measures serve to standardize the parental age of onset as determined by the "age of first progressive cognitive decline", the information may be influenced by recall bias.

Figure S1: Parental Age of Onset Form



DIAN
Dominantly Inherited
Alzheimer Network

ID: _____ Date: _____ Initial Visit

Age at Onset Evaluation

Evaluation Date: _____

DIAN Participant ID: _____ Informant's DIAN ID #: _____

☐ Check here if an approved proxy is being used to determine AAO.
**A proxy or substitute first degree relative may be used to complete this form. The proxy must be designated by the DIAN Coordinating Center. Please complete an Exceptions Form and fax the family pedigree to the DIAN Global Clinical Coordinator at (314) 286-2433.*

Affected Parent's (or approved Proxy) DIAN ID #: _____

Instructions:

Age at Onset (AAO) operational definition: The age at onset is defined as the age when the *first progressive decline* in cognition, behavior, or motor function was noted.

Calculation: AAO of the affected parent is calculated using the answer given for question #2 on the Age at Onset Evaluation Form ("Year of onset of first progressive symptom") minus the parent's year of birth.

The clinician determines the AAO after consulting at least one reliable collateral source, the participant, and any other sources of information (e.g. medical records, peripheral family members). Cognitive decline accompanied by minor functional change in the individual's abilities (e.g. in judgment, personal finances, home activities, orientation) which arouse caregiver curiosity or concern help to determine AAO. To obtain the most accurate information, encourage informants to think of changes in relation to other documented/validated dates such as birthdates, weddings, deaths, and other major events. Use the questions and prompts on the next page to help determine the age at onset.

Use of Alternate Codes: If the site research team is unable to provide the requested information, please use the most appropriate code:

- **Not Applicable (NA):** Use this code if the affected parent or proxy was known not to experience the symptom/deficit or is not currently experiencing the symptom/deficit.
- **Unknown (UNK):** Use this code if it is unknown if the symptom or deficit existed.

** If it is known that the affected parent or proxy experienced a symptom or deficit, a year MUST be provided.**

Age of Dementia Diagnosis operational definition: Record the year at which the affected parent or approved proxy met the DSM-IV or NINCDS-ADRDA diagnostic criteria for dementia in question #3. This is defined as the year at which the individual displayed cognitive deficits causing impairment in social or occupational functioning that represented a significant decline from a previous level of functioning. This may differ from the year that dementia was diagnosed by a physician.



ID: _____

Date: _____

Initial Visit

Worksheet:

1. Year last known to be normal and without deficits: _____

2. Year of onset of first progressive symptom: _____ *

**Use this year to calculate the Age at Onset. The parental AAO is recorded on the Participant Family History Form, the Participant Pedigree, and the Visit Frequency Calculation Form.*

Year of onset for the following deficits (must provide year if present):

Memory/Cognition _____ / NA / UNK

Behavior _____ / NA / UNK

Motor Function _____ / NA / UNK

3. Year that evaluator thinks person met NINCDS-ADRDA or DSM-IV diagnostic criteria for Alzheimer's disease: _____ / NA / UNK

4. Year of death: _____ / NA / UNK

Cause of death: _____

Autopsy confirmed: ____ Yes ____ No ____ Unknown

Estimated CDR at death (Circle): 0.5 1 2 3

Questions and prompts to determine age at onset:

1. What year was the last time the individual was completely normal with no impairment in activities (job/hobbies)?
2. In retrospect, what year was the first time changes were noticed (compared to this person's baseline function)?
 - a. Recall the first time this individual manifested forgetfulness.
 - b. Recall the first time this individual had impairment in judgment (e.g. in planning activities).
 - c. Recall the first time this individual displayed a change in temporal or physical orientation (confusion regarding dates or locations).
 - d. Recall first time any changes in behavior or cognition were noticed.
 - e. Did these changes continue to progress from that time forward?
3. What year did the individual meet criteria for dementia of the Alzheimer's type as defined above?
4. Is this individual living?
 - a. If deceased, was there an autopsy of the brain? If yes, what were the results?
 - b. What was the stage of dementia at death? Use the CDR table below to estimate.

Figure S2: Comparison by EYO with 95% CI

Comparison of clinical, cognitive, structural, metabolic, and biochemical changes by estimated years to symptom onset with 95% confidence interval bands. The normalized differences between mutation carriers and non-carriers are shown versus EYO and plotted with a LOESS curve. The order of differences suggest decreasing CSF A β 42, followed by fibrillar A β deposition, then increased CSF tau, followed by hippocampal atrophy and hypometabolism before cognitive and clinical changes. Mild dementia (CDR 1) is indicated to occur at an average of 3.3 EYO.

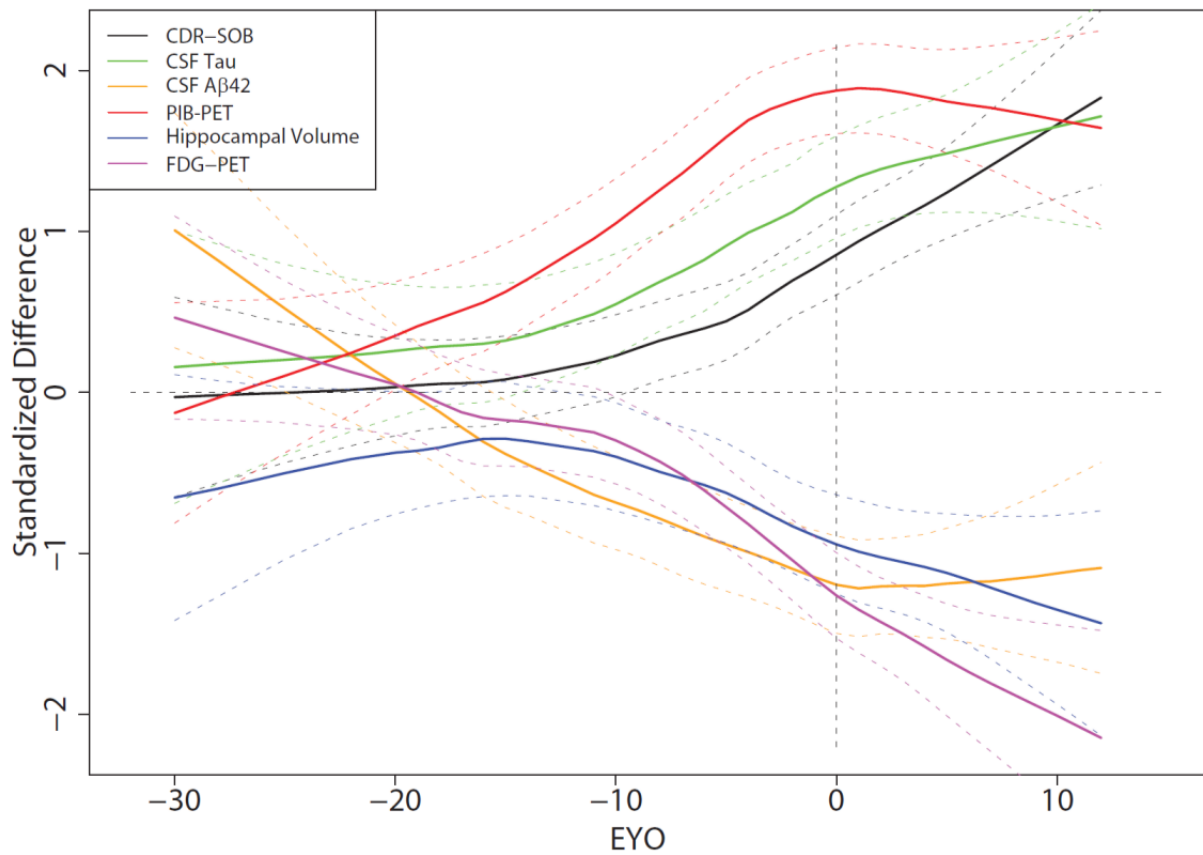


Table S1: DIAN participant characteristics

Characteristics of participants from the DIAN cohort (p-value of Non-carrier vs. Asymptomatic Carrier). Asymptomatic carriers are defined as CDR 0 (normal cognition) and symptomatic carriers are defined as CDR >0 (cognitive impairment). One non-carrier was symptomatic with a CDR of 0.5. The clinical presentation as reported by the clinician judgment of symptoms (UDS B9 form) is most commonly memory disturbance (32 of 42 first symptom presentation) followed by Judgment and Problem Solving (4 of 42) and Attention/Concentration (4 of 42).

Characteristic	Non-carrier	Asymptomatic Carrier	Symptomatic Carrier	p-value
No. (%)	40 (31.2)	45 (35.2)	43 (33.6)	0.2941
Age, mean (SD) yr	39.5 (8.9)	33.6 (7.8)	44.8 (9.5)	0.0021
Education Level, mean (SD) yr	15.0 (2.5)	14.6 (2.6)	13.2 (2.2)	0.7225
ApoE ε4 +, no. (%)	9 (22.5)	9 (20)	13 (26.5)	0.7297
Male Sex, no. (%)	17 (42.5)	16 (35.6)	20 (46.5)	0.4641

Table S2: Comparison of *PSEN1* to *PSEN2* and *APP*

In order to compare the effects of mutation gene type on the findings, *PSEN2* (n=11) and *APP* (n=7) were collapsed into a comparison group. Analyses were performed for all measures comparing non-carriers to *PSEN1* (n=70) to the *PSEN2/APP* group. All p-values were >0.05, indicating no statistically significant difference between *PSEN1* and other mutation types.

Marker	P-value range for comparison of <i>PSEN1</i> to <i>PSEN2</i> and <i>APP</i>
CDR-SOB	0.57 -- 0.99
MMSE	0.66 -- 0.97
Logical Memory	0.47 -- 0.89
PIB PET	0.06 -- 0.93
FDG PET	0.12 -- 0.52
MRI Hippocampal	0.57 -- 0.93
CSF A β 42	0.68 -- 0.95
CSF tau	0.23 -- 0.85
Plasma A β 42	0.76 -- 0.99

Table S3: Interaction terms of model.

The terms in each model of all possible 2-factor, 3-factor interaction terms along with second and third order EYO terms were examined to reach a final model that fitted the data well for each marker. The presence of an ApoE $\epsilon 4$ allele was tested in the model and there is no significant ApoE $\epsilon 4$ effect.

Marker	Terms in the model for Table 2
CDR-SOB	Mutation, EYO, Mutation*EYO, EYO ² , Mutation*EYO ²
MMSE	Mutation, EYO, Mutation*EYO, EYO ² , Mutation*EYO ²
Logical Memory	Mutation, EYO, Mutation*EYO, EYO ² , Mutation*EYO ² , EYO ³
PIB	Mutation, EYO, Mutation*EYO, EYO ² , Mutation*EYO ² , EYO ³ , Mutation*EYO ³
FDG	Mutation, EYO, Mutation*EYO
MRI Hippocampal	Mutation, EYO, Mutation*EYO
CSF A β 42	Mutation, EYO, Mutation*EYO, EYO ² , Mutation*EYO ²
CSF tau	Mutation, EYO, Mutation*EYO
Plasma A β 42	Mutation, EYO, Mutation*EYO, EYO ²

Table S4: Number of participants by EYO

The number of participants in each 5 year bin compared is listed with the percentages.

5 Year Bin	Counts	Percentage (%)
-30 ~ -25	7	5.51
-25 ~ -20	6	4.72
-20 ~ -15	15	11.81
-15 ~ -10	18	14.17
-10 ~ -5	21	16.54
-5 ~ 0	25	19.69
0 ~ 5	13	10.24
5 ~ 10	12	9.45
10 ~ 15	8	6.30
15 ~ 20	2	1.57

Author contributions:

This manuscript has been reviewed by DIAN Study investigators for scientific content and consistency of data interpretation with previous DIAN Study presentations and publications.

The DIAN steering committee composed of the principal investigator at each clinical site, DIAN core leaders, and the project scientists from the sponsor (NIA), designed and implemented the study. The ADCS and the DIAN Informatics Core accumulated data in a central database during the study and the DIAN Biostatistics and Clinical Cores performed data analyses. The study investigators, who had access to all data analyses and wrote the manuscript, attest to the veracity and completeness of the data and the fidelity of the study to the protocol. R.J.B. wrote the first draft of the manuscript. The decision to submit the manuscript for publication was made by the investigators.

Supplementary References

1. Cruchaga C, Chakraverty S, Mayo K, et al. Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. *PloS one* 2012;7:e31039.
2. Cruchaga C, Kauwe JS, Mayo K, et al. SNPs associated with cerebrospinal fluid phospho-tau levels influence rate of decline in Alzheimer's disease. *PLoS genetics* 2010;6.
3. Fischl B, van der Kouwe A, Destrieux C, et al. Automatically parcellating the human cerebral cortex. *Cereb Cortex* 2004;14:11-22.
4. Buckner RL, Head D, Parker J, et al. A unified approach for morphometric and functional data analysis in young, old, and demented adults using automated atlas-based head size normalization: reliability and validation against manual measurement of total intracranial volume. *Neuroimage* 2004;23:724-38.
5. Mintun MA, Larossa GN, Sheline YI, et al. [11C]PIB in a nondemented population: potential antecedent marker of Alzheimer disease. *Neurology* 2006;67:446-52.
6. Eisenstein SA, Koller JM, Piccirillo M, et al. Characterization of extrastriatal D2 in vivo specific binding of [18F](N-methyl)benperidol using PET. *Synapse* 2012:n/a-n/a.
7. Rowland DJ, Garbow JR, Laforest R, Snyder AZ. Registration of [18F]FDG microPET and small-animal MRI. *Nuclear medicine and biology* 2005;32:567-72.
8. Cleveland S, Grosse E, Shyu WM. *Local Regression Models: Statistical models in S*. Pacific Grove, Calif.: Wadsworth & Brooks/Cole Advanced Books & Software; 1992.
9. Talairach J, Tournoux P. *Co-planar stereotaxic atlas of the human brain : 3-dimensional proportional system : an approach to cerebral imaging*. Stuttgart ; New York: Georg Thieme; 1988.
10. Littell RC. *SAS system for mixed models*. Cary, N.C.: SAS Institute Inc.; 1996.
11. Ihaka R, Gentleman R. R: A Language for Data Analysis and Graphics. *Journal of Computational and Graphical Statistics* 1996;5:299-314.